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14. ABSTRACT

Our aim in Year 1 was to optimize and validate conditions for maintaining the structure and function of prostate tissue slice cultures (TSCs) in an MRS-compatible, 3-D tissue culture bioreactor. In conjunction with our collaborators at UCSF, conditions have been identified in which as few as 4 TSCs, from normal or malignant tissue, can be placed in an MRS-compatible bioreactor and remain metabolically active for at least 24 hours. Histological analysis of recovered TSCs indicated good maintenance of structure.

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INTRODUCTION

This project involves the lab of Dr. Donna Peehl at Stanford and Drs. Kurhanewicz and Ronen at UCSF. Overall, our goal is to identify hyperpolarized molecular imaging biomarkers for improved prostate cancer patient-specific treatment planning and early assessment of response to hormonal and chemotherapy. Specifically, Dr. Peehl's role is to supply prostate tissue cultures and develop optimal methods of culture. Our project has three aims: (1) to optimize and validate conditions for maintaining the structure and function of prostate tissue slice cultures (TCSs) in an nuclear magnetic resonance (NMR)-compatible, 3-dimensional tissue culture bioreactor, (2) to use the TSC/NMR bioreactor model to identify hyperpolarized metabolic biomarkers of prostate cancer presence and aggressiveness, and (3) to use the TSC/NMR bioreactor model to identify hyperpolarized metabolic biomarkers of response to hormone and chemotherapy. Our goal was to achieve Aim 1 in Year 1 and we have made significant progress toward this goal.

BODY

Our first designated task was to vary basal medium, growth and differentiation factors, hormones, tissue perfusion and oxygen levels. For ease, we use commercially-available medium DMEM without glucose or sodium pyruvate, which is then supplemented with glucose (1 g/L) and all of the growth factors and hormones that are in our serum-free medium formulation for human prostate epithelial cell culture [1].

While DMEM appears to suffice as a basal medium, we have explored modifications of our own medium, PFMR-4A [1]. The advantage of this medium is that we make it ourselves and therefore in the future we can manipulate the ingredients and investigate effects on metabolism. We have eliminated the only undefined growth supplement, bovine pituitary extract (BPE), and evaluated the effect on maintenance of structure of TSCs in fixed and H&E-stained sections. TSCs maintained well for 24 hours in medium without BPE, although signs of cellular disintegration appeared by 48 hours. This result suggests that BPE could be eliminated for short-term spectral analysis in the bioreactor.

We also explored the possibility of freezing TSCs after preparation. The ability to freeze and recover TSCs would be very helpful, since sometimes the logistics of surgical schedules and NMR availability are complicated. Based on a report that claimed that viable colon tissues could be recovered after freezing, we followed the published protocol and recovered TSCs after freezing. We found very poor recovery, with massive cellular degradation. It does not appear that viable TSCs can be recovered after freezing, at least by this protocol.

Alternatively, we tested culture at 4°C overnight as another option to preserve TCSs for delayed analysis by NMR. This experiment was obviously performed in air rather than in the 5% CO_2/95% air ambience of the culture incubator, but tissue histology after 24 hours was surprisingly good. We will continue to explore the possibility of maintaining TSCs at 4°C prior to spectral analysis in the bioreactor.

One other modification we tested was the addition of N-acetylcysteine (NAC) to the medium. NAC protects cells from oxidative damage, and it is possible that our inability to maintain TSCs for longer than 72 hours in culture might be due to oxidative damage. In particular, NAC was

found to prevent the loss of luminal cells in matrix-deprived breast epithelial cells [2], and it is in the luminal epithelial cell layer that we observe the most cell loss in prostate TSCs over time. We evaluated TSCs from a number of cases in medium +/- NAC, and it is our impression that cellular viability is improved in the presence of NAC. In future experiments, we will determine how the presence or absence of NAC effects metabolic labeling in the bioreactor.

Our second designated task was to evaluate maintenance of structure and function by histopathology and immunohistochemistry. Cores (8-mm diameter) of fresh tissue are bored aseptically from fresh prostate specimens. When the tissues are sliced at 300-um by the Krumdieck microtome, we freeze or fix every 5th section to confirm the histopathology of the intervening sections to be used for metabolic imaging. These frozen or fixed tissue sections also serve as a reference for structure and function at t_0. For proton labeling, the tissues are put into culture for two hours in medium containing ^{13}C-glucose. The tissue sections are then recovered and snap-frozen. Conditioned medium is also collected and frozen. These materials are then shipped to UCSF for metabolic analysis. For hyperpolarized labeling, the TSCs are placed in medium on ice and immediately transported to UCSF. TSCs recovered after culture are stained with H&E or with cell type-specific markers by immunohistochemistry to evaluate maintenance of structure and function after manipulations.

Our third designated task was to evaluate metabolic integrity by sequential ^{31}P spectroscopy and dynamic hyperpolarized ^{13}C spectroscopy. We discovered that the antibiotic gentamicin could not be included in the medium because its peak interferes with ^{31}P spectral analysis. Similarly, we cannot slice the tissues in HEPES-buffered saline (HBS), as we typically do, because HEPES also causes spectral interference. Accordingly, we now slice in phosphate-buffered saline (PBS). We have supplied our collaborators at UCSF with a number of cases of paired normal/malignant TSCs and a manuscript describing the results of ^{31}P spectroscopy is in preparation. Overall, our results indicate that normal and malignant TSCs express metabolic phenotypes previously observed in the prostate. In addition, a number of pilot studies have been conducted to develop optimal conditions for evaluation of TSCs by dynamic hyperpolarized ^{13}C spectroscopy. Our collaborators at UCSF have engineered a very small bioreactor that requires only four or possibly fewer TSCs for analysis. This is a significant development because it will enable us to obtain sufficient tissue from even small cancers, and we are more likely to be able to supply TSCs containing a high proportion of cancer in the malignant TSCs (prostate cancer always has admixed benign glands).

In summary, we have accomplished what we set out to do in Year 1 and are well-positioned to carry out Aims 2 and 3 in the next two years.

KEY RESEARCH ACCOMPLISHMENTS

- **Identified a basal medium, DMEM, that when supplemented with growth factors and hormones included in the formulation for our prostate-specific medium, PFMR-4A, maintains structure and function of normal and malignant prostate TSCs compatible with metabolic profiling**

- **Obtained preliminary data that TSCs might be preserved for delayed metabolic profiling by culturing at 4° C, facilitating complicated logistics of tissue procurement and spectral studies**
- **Obtained preliminary data that an anti-oxidant, N-acetylcysteine, might extend longevity of TSCs**
- **Identified and removed factors in the medium or buffers that interfere with spectral analyses**
- **Provided sufficient sets of normal and malignant TSCs to USCF to complete our ^{31}P spectroscopic analyses for publication**

REPORTABLE OUTCOMES

None.

CONCLUSIONS

We achieved the three components of our first aim. TSCs can be maintained in an NMR-compatible bioreactor for spectral analysis, providing the desired platform to proceed with Aims 2 and 3.

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APPENDICES

None.